REMARKS

In the parent application (serial no. 09/922,501), Applicants were given a 28 way restriction. Groups I – VI were drawn to nucleic acid molecules of SEQ ID NOS: 1, 3, 5, 7, 9, and 11 or encoding SEQ ID NOS: 2, 4, 6, 8, 10 and 12, respectively, vectors containing the nucleic acid molecules, bacterial cells containing the vectors, oligonucleotides and methods of using the nucleic acids. Groups VII - XII were drawn to a polypeptide and groups XIII – XVIII were drawn to antibodies specific for the polypeptide sequences. Applicants elected Group IV with claims drawn to SEQ ID NO: 7 and SEQ ID NO: 8 (prmA). In the present application, Applicants have amended the claims which are now drawn to SEQ ID NO: 11 and SEQ ID NO: 12 (yiaX2). These sequences correspond to the restricted claims of Group VI in the parent application.

With entry of the instant amendment, claims 5 - 7, 11 - 16, 20 - 27, 36 - 40 and 49 - 56 are pending. Claims 5 - 7, 11, 13, 16, 22, 23, 25 and 36 have been amended and claims 49 - 56 are new. Claims 1 - 4, 8 - 10, 17 - 19, 28 - 35 and 41 - 48 have been canceled. New matter has not been introduced by the present amendment. While Applicants have provided a copy of the amendments to the claims as required by the revised rules (37 CFR 1.121), a clean copy of the pending claims has also been provided for the convenience of the Examiner.

Amended claim 5 has been rewritten in independent form and recites a nucleic acid molecule encoding a polypeptide having the sequence of SEQ ID NO: 12 or an amino acid sequence having at least 40% sequence identity thereto, wherein said polypeptide is a transmembrane protein which has 2,5-diketo-gluconate (2,5-DKG) permease activity. Support is found in the original claims and at page 5 of the disclosure. Claims 6, 7 and 16 have been amended to recite SEQ ID NO: 12. Claim 11 has been rewritten in independent form. Claims 22 and 23 have been amended to further comply with section 112 as to form. Claims 25 and 36 have been amended to provide antecedent basis for the abbreviation 2-KLG. Claim 36 has further been amended to clarify that the nucleic acid encoding the polypeptide having 2,5-DKG permease activity is introduced into a bacterial host and that the bacterial host is cultured under suitable conditions to produce 2-KLG. Additionally a change in dependency from claim 1 to claim 5 has been made.

New claim 49 defines the host cells as *E. coli*. New claim 50 defines the nucleic acid molecule used in claim 36 as encoding a polypeptide having at least 80 % sequence identity to SEQ ID NO: 12 and new claim 51 defines the nucleic acid molecule used in claim 36 as having at least 95% sequence identity with the sequence set forth in SEQ ID NO: 11.

New claim 52 is directed to a method for increasing the transport of 2, 5-DKG across a cell membrane comprising a) introducing the nucleic acid molecule of claim 5 having 2,5-DKG permease activity into a bacterial host cell, b) allowing expression of the 2,5-DKG permease and c) culturing the bacterial host cell under suitable conditions for the transport of 2,5-DKG. Support is found *inter alia* at page 10 of the disclosure. Claims 53 - 55 depend from claim 53. Claim 53 is directed to a host cell selected from the group of *E. coli*, *Pantoea* or *Klebsiella* cells, and support is found at pages 24 - 26 of the disclosure. Claim 54 is directed to a nucleic acid molecule which encodes a polypeptide having at least 80 % sequence identity to SEQ ID NO: 12, and claim 55 defines the nucleic acid molecule as having at least 95% sequence identity with the sequence set forth in SEQ ID NO: 11. Support is found in the original claims.

Independent new claim 55 is directed to using part of the nucleic acid molecule of SEQ ID NO: 11 has a probe under stringent hybridization conditions to identify further 2,5-DKG permeases and support is found in original claim 28 and at pages 17 - 19 and 28 and 29 of the disclosure.

With respect to SEQ ID NOs: 11 and 12, it is stated in the disclosure at page 13,

> "Specifically with respect to an isolated nucleic acid molecule containing the nucleotide sequence designated SEQ ID NO: 11 or encoding the *yia*X2 polypeptide designated SEQ ID NO: 12, the term "isolated" is intended to mean that the nucleic acid molecule does not contain any of the flanking open reading frames (orfs) present in the *K. oxytoca yia* operon, such as the orfs designated *lyx*K and *orf*1, described in WO 00/22170. "

Also as stated in the examples at page 42,

> "WO 002170 describes the identification and sequencing of an operon from *Klebsiella oxytoca,* designated the *yia* operon, which contains eight putative open reading frames. Because disruption of this operon abolished the ability of *K. oxytoca* to utilize ascorbic acid as the sole carbon source, the *yia* operon was predicted to be involved in the catabolism of ascorbic acid. The functions of the polypeptides encoded by the individual open reading frames in the *yia* operon were not described in WO 00/22170."

Applicants have attached a copy of this publication for the Examiner's convenience.

Applicants have amended the specification to correct the provisional application serial numbers and to include the priority information of the parent application, which has now been allowed.

Applicants assert claims 5 - 7, 11 - 16, 20 - 27, 36 - 40 and 49 - 56 are in condition for allowance.

Respectfully submitted,

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Enc. Clean copy of pending claims
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APPENDIX - Clean Copy of the Pending Claims:

5. (Currently amended): An isolated nucleic acid molecule which encodes a polypeptide having an amino acid sequence of SEQ ID NO: 12 or an amino acid sequence having at least 40% sequence identity thereto, wherein said polypeptide is a transmembrane protein which has 2,5-diketo-gluconate (2,5-DKG) permease activity.

6. (Currently amended): The isolated nucleic acid molecule of claim 5, comprising a nucleotide sequence which encodes a polypeptide having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 12.

7. (Currently amended): The isolated nucleic acid molecule of claim 5, which encodes a polypeptide having the amino acid sequence of SEQ ID NO: 12.

11. (Currently amended): An isolated nucleic acid molecule comprising a polynucleotide which encodes a polypeptide having an amino acid sequence of SEQ ID NO. 12 or an amino acid sequence having at least 40% sequence identity thereto, wherein said polypeptide has 2,5-diketo-gluconate (2,5-DKG) permease activity, and wherein the polynucleotide is operatively linked to a promoter of gene expression.

12. (Original): The isolated nucleic acid molecule of claim 11, wherein said promoter is a *lac* promoter.

13. (Original): A vector comprising the isolated nucleic acid molecule of claim 11.

14. (Original): The vector of claim 13, comprising a spectromycin resistance gene.

15. (Original): A bacterial cell, comprising the vector of claim 13.

16. (Currently amended): The bacterial cell of claim 15, wherein said isolated nucleic acid molecule comprises a nucleotide sequence which encodes a polypeptide having an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO: 12.

20. (Original): The bacterial cell of claim 15, which is of the genus *Klebsiella*.

21. (Original): The bacteria cell of claim 15, which is deficient in endogenous 2,5-DKG activity.

22. (Currently amended): The bacterial cell of claim 21, further comprising an isolated nucleic acid molecule encoding a polypeptide having 2-keto reductase activity and at least 80% sequence identity to SEQ ID NO: 14.

23. (Currently amended): The bacterial cell of claim 21, further comprising an isolated nucleic acid molecule encoding a polypeptide having 5-keto reductase activity and at least 80% sequence identity to SEQ ID NO: 16 .

24. (Original): The bacterial cell of claim 15, which is of the genus *Pantoea*.

25. (Currently amended): The bacterial cell of claim 15, which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-keto-L-gulonic acid (2-KLG) .

26. (Original): The bacterial cell of claim 25, which expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

27. (Original): The bacterial cell of claim 26, which is deficient in endogenous 2-keto-reductase activity.

36. (Currently amended): A method of using the isolated nucleic acid molecule of claim 5 to enhance 2-keto-L-gulonic acid (2-KLG) production, comprising

a) introducing the isolated nucleic acid molecule of claim 5 into a bacterial cell which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-KLG,

b) allowing expression of the polypeptide encoded by said nucleic acid molecule and

c) culturing the bacterial cell under suitable conditions to produce 2-KLG.

37. (Original): The method of claim 36, wherein said bacterial cell further expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

38. (Original): The method of claim 37, wherein said bacterial cell is deficient in endogenous 2-keto reductase activity.

39. (Original): The method of claim 36, wherein said bacterial cell is of the genus *Pantoea*.

40. (Original): The method of claim 36, further comprising converting said 2-KLG to ascorbic acid.

49. (New): The bacterial cell of claim 15, which is an *E. coli* cell.

50. (New): The method of claim 36, wherein the nucleic acid molecule encodes a polypeptide having at least 80% sequence identity to SEQ ID NO: 12.

51. (New): The method of claim 36, wherein the nucleic acid molecule has the sequence of SEQ ID NO: 11 or a sequence having at least 95% sequence identity thereto.

52. (New): A method for increasing the transport of 2, 5-DKG across a cell membrane into a bacterila host cell comprising

a) introducing the nucleic acid molecule of claim 5 having 2,5-DKG permease activity into a bacterial host cell,

b) allowing expression of the 2,5-DKG permease, and

c) culturing the bacterial host cell under suitable conditions for the transport of 2,5-DKG into the bacterial host cell.

53. (New): The method according to claim 52, wherein the bacterial host cell is an *E. coli*, *Pantoea* or *Klebsiella* host cell.

54. (New): The method according to claim 52, wherein the nucleic acid molecule encodes a polypeptide having at least 80 % sequence identity to SEQ ID NO: 12.

55. (New): The method according to claim 52, wherein the nucleic acid molecule has the sequence of SEQ ID NO: 11 or a sequence having at least 95% sequence identity thereto.

56. (New): An isolated oligonucleotide comprising at least 20 contiguous nucleotides of the nucleotide sequence of SEQ ID NO: 11, wherein said oligonucleotide is used as a probe and hybridizes under stringent hybridization conditions to a nucleic acid that encodes a polypeptide having 2,5-diketo-D-gluconic acid permease activity.